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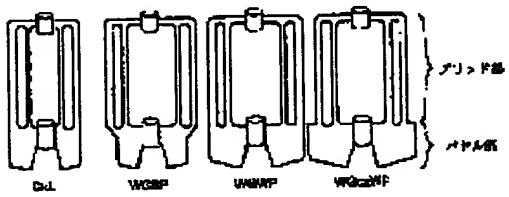
(54) GATE TYPE BLADE FOR AGITATING HIGH NON-NEWTONIAN FLUID

(57)Abstract:
PROBLEM TO BE SOLVED: To use a gate type blade on aerating agitation to obtain a high oxygen capacity coefficient by making the ratio of the blade diameter in a grid part of an agitator equipped with a gate type blade to the tank inner diameter be a specified value or more.
SOLUTION: The ratio of the blade diameter in a grid part of a agitator equipped with a gate type blade to the tank diameter is made to be ≥0.6, preferably ≥0.65. And of gate type blades, it is better that a bottom paddle part or a bottom turbine part is integrated into a grid part, and that the blade diameter of a bottom part or a bottom turbine part is smaller than that of a grid part. In this way, in this case a fluid having high non-Newtonian property is agitated, for example, when cellulose producing bacteria are aeration agitated and cultured, the gate type blade is particularly profitably used.



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CLAIMS

[Claim(s)]
[Claim 1] Stirring equipment equipped with the gate type wing characterized by the ratio to the tub bore of the wing diameter in the grid section being 0.6 or more.
[Claim 2] Stirring equipment equipped with the gate type wing according to claim 1 characterized by the wing diameter in the bottom paddle section or the bottom turbine section being smaller than the wing diameter in the grid section.
[Claim 3] Stirring equipment according to claim 1 or 2 for using it for stirring of the high fluid of the non-Newton nature.
[Claim 4] Stirring equipment according to claim 1 or 2 for using it for culture of a cellulose production bacillus.
[Claim 5] How to carry out aeration spinner culture of the cellulose production bacillus, using stirring equipment according to claim 1 or 2 as a fermenter, and to manufacture the cellulose nature matter.
[Claim 6] Use to culture of the cellulose production bacillus of stirring equipment according to claim 1 or 2.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention cultivates the fungus body belonging to the microorganism (henceforth a "cellulose production bacillus") which has the capacity to produce the cellulose nature matter using the stirring equipment equipped with the gate type wing which is so-called "gate type wing", and has the description in the configuration which has opening which forms a grid in a monotonous aerofoil, and this equipment, and relates to the approach of manufacturing the cellulose nature matter (henceforth "bacterial cellulose", or "BC").
[0002]
[Description of the Prior Art] since BC (bacterial cellulose) is edible, and is used in the food field and also it is excellent in drainage system dispersibility — maintenance of the viscosity of food, cosmetics, or a coating, and a food raw material — there is industry top utility value as strengthening of the ground, maintenance of moisture, the improvement in food stability, a low-calorie-content additive, or an emulsification stabilization assistant. BC is characterized by the fragment width of face of fibril being small about 2 figures compared with the cellulose manufactured from wood pulp etc. Therefore, the disaggregation object of BC has various kinds of industrial applications as a reinforcing agent for a macromolecule, especially drainage system macromolecules based on the structural physical description which microfibril requires. Since a high modulus of elasticity in tension is shown, the outstanding mechanical characteristic based on the structural description of microfibril is expected, and the matter which solidified such a cellulose nature disaggregation object the shape of paper and in the shape of solid has the application as various industrial materials.
[0003] About the manufacture approach of BC, JP,62-265990,A, JP,63-202394,A, JP,6-43443,B, etc. have the publication about the manufacture approach of BC. It consists of a carbon source, a peptone, a yeast extract, sodium phosphate, and a citric acid as a nutrition culture medium made suitable in case a cellulose production bacillus is cultivated. Schramm/Hestrin The culture medium (Schramm et al., J.General Biology, II, pp.123-129, and 1954) is known. Moreover, it is a cellulose generation promoter according to the specific nutrient in a culture medium to such a nutrition culture medium. Add an inositol, phytic acid, a pyrrolo quinoline quinone (PQQ) (JP,5-1718,B; Mitsuo Takai, Japan Technical Association of the Pulp and Paper Industry, the 42nd volume, No. 3, the 237-244th page), etc., or Furthermore, it is found out by adding a carboxylic acid or its salt (Japanese Patent Application No. No. 191467 [five to]), an invertase (Japanese Patent Application No. No. 331491 [five to]), and a methionine (Japanese Patent Application No. No. 335764 [five to]) that the productivity of the cellulose nature matter improves. Moreover, the method of cultivating a cellulose production bacillus under the conditions of the oxygen-transfer coefficient (kL a) of the specific range is also proposed (Japanese Patent Application No. No. 31787 [seven to]). Furthermore, the method of cultivating a cellulose production bacillus is also proposed, maintaining the internal pressure of a fermenter more than fixed (Japanese Patent Application No. No. 276408 [seven to]). Moreover, as a culture format of cultivating a microorganism, standing, shaking, or aeration spinner culture has been used conventionally. Moreover, as culture operation information, the so-called batch fermentation method, the fed-batch-fermentation method, the repetitive batch fermentation method, the continuous fermentation method, etc. have been used. In addition, as a stirring means, pump drive circulation of an impeller (impeller), an air lift fermenter, and fermentation broth, the combination of these means, etc. are used, for example. As a class of impeller, the gate type wing, the turbine blade, the helical ribbon wing, the screw wing, etc. are known.
[0004] The oxygen demand of culture is made to satisfy by aeration and stirring in a industrial general fermentation process generally. However, according to many fermentation processes, it is thought important to examine the factor which rate-limiting [of the productivity] is carried out by the oxygen supply ability of a fermenter, therefore affects oxygen supply on the occasion of culture of a microorganism. It faces that the oxygen in air moves to a fungus body by the culture system, and the oxygen transfer from air bubbles to the liquid phase is represented by the degree type.
[Equation 1] dCL/dt = kL a(C*-CL) = HkL a(PG-PL) dCL / dt : Oxygen transfer rate (mmol/L-hr)
kL a: Oxygen-transfer coefficient (hr-1)
CL : Dissolved oxygen concentration in culture medium (mmol/L)
C* : Dissolved oxygen concentration [**** / oxygen tension / of air bubbles] (mmol/L)
H : Henry constant PG : Oxygen tension in a gaseous phase (it will rise, if it pressurizes)
PL : Oxygen tension in the liquid phase [0005]
[Problem(s) to be Solved by the Invention] Now, the equipment equipped with the gate type wing which unified the bottom paddle section and the grid section as a stirred tank excellent in various stirring properties from the former is the brand name "the Max blend" (Sumitomo Heavy Industries, Ltd.), and it is **********. However, there is no example which the property which was excellent in this stirred tank was evaluated using the low simulation liquid of non-Newton nature like a carboxymethyl cellulose (CMC), and was actually evaluated with the high liquid of non-Newton nature like BC. When the non-Newton nature of a solution is approximated with the exponential-function model (Power Law model) shown below It is expressed with Power Law Index (n), and change of the apparent viscosity to an average shear rate can say greatly that the non-Newton nature is high, so that this value is small.
[Equation 2]

$$\eta_{ap} = K \mid \dot{\gamma} \mid^{(n-1)}$$

etaap is an apparent viscosity and K. consistency index, [External Character 1]

$\dot{\gamma}$

a ****** shear rate and n — Power law index it is . n determines that the variation in K in each shear conditions becomes min. Incidentally, to 0.8, xanthan gum of BC is [this (n) value / CMC] very as small as 0.1 to 0.3, and the suspension or the culture medium of BC is understood that the non-Newton nature is high.

[0006] Although are desirable, therefore it is generally considered that the large-sized wing is suitable in mixing by the high fluid of the non-Newton nature that the distance of a fluid and a wing is small since change of the apparent viscosity to a shear is large, a large-sized wing has the weak shearing force over consumption power, and to be unsuitable is considered by the shear of air bubbles required for oxygen transfer. Moreover, depending on the discharge flow which the shear of the air bubbles in the bottom paddle section near a sparger or the bottom turbine section is important, and is too strong although it is also expectable that the whole fluidity improves by the discharge flow of this part, the lump of air arises near the wing, and we are anxious also about possibility of reducing a fluidity conversely. In order to raise oxygen transfer in the high fluid of the non-Newton nature until now, there is no example which examined the wing configuration near the sparger. When stirring equipment equipped with the gate type wing which carried out the specific configuration was used on the occasion of aeration stirring to for example, BC suspension or BC culture medium based on the above-mentioned recognition as a result of research of the oxygen transfer in the high fluid of the non-Newton nature, and production by fermentation, this invention person etc. found out that a high oxygen capacity coefficient (kL a) was obtained, found out that high productivity was acquired also in culture, and completed this invention.

[0007]

[Means for Solving the Problem] That is, this invention relates to stirring equipment equipped with the gate type wing by which the ratio (d/D) to the tub bore of the wing diameter in the grid section is characterized by being 0.65 or more preferably 0.6 or more. The side face of the grid section may incline and it considers as the ratio of the wing diameter of the grid section in the minimum width of face in that case. In the gate type wing of this invention, it is [direction] desirable and the bottom paddle section or the bottom turbine section has a more desirable thing with those wing diameters (d (P)) smaller than a wing diameter [in / it is uniting with the grid section and / the grid section] (d). The stirring equipment of this invention can be especially used advantageously, in case aeration spinner culture of the cellulose production bacillus is carried out when stirring the high fluid of the non-Newton nature for example. In addition, this contractor can choose suitably the rate that the configuration and the number of the point of others about the structure and the configuration of the gate type wing of this invention, for example, the configuration and number of a grid, the bottom paddle section, or the bottom turbine sections, and a paddle aspect product occupy, the thickness of an aerofoil, etc., according to the purpose etc. Moreover, it faces enforcing this invention approach and, in addition to above-mentioned culture format and culture operation information, "said approach characterized by to be the manufacture approach of the cellulose nature matter of circulating the culture medium which contains a fungus body among decollators, such as a culture apparatus, a floatation unit, and a wedge filter, and to separate the cellulose nature matter which is a product from a fungus body and culture medium in this decollator" indicated by Japanese Patent Application No. No. 192287 [six to] can also be taken.

[0008] The cellulose production bacillus used in this invention For example, Acetobacter xylinum subsp. sucrofermentans represented by 2001 shares of BPR (Acetobacter xylinum subsp.sucrofermentans), Acetobacter xylinum () [Acetobacter] xylinum ATCC23768, Acetobacter xylinum ATCC23769, Acetobacter pasteurianus (A.pasteurianus) ATCC10245, Acetobacter xylinum ATCC14851, Acetobacter xylinum ATCC11142 To and the acetic bacteria of Acetobacter xylinum ATCC10821 grade and others Agrobacterium, Rhizobium, the Sarcina, Pseudomonas, They are the various variants invented by Achromobacter, Alcaligenes, an Aerobacter group, an azotobacter group, and the ZUGUREA group list by carrying out variation processing of them by the well-known method of using NTG (nitrosoguanidine) etc. In addition, 2001 shares of BPR is deposited with the Ministry of International Trade and Industry National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology patent microorganism deposition pin center,large on February 24, Heisei 5 (trust number FERM P-13466), and the management of it is transferred to the deposition (trust number FERM BP-4545) based on Budapest Treaty about international acknowledgement of the deposition on a patent procedure on February 7, 1994 after that.

[0009] the chemical variation art using variation agents, such as NTG, — Bio Factors, Vol.1, and p.297-302 — and (1988) — J.Gen.Microbiol, Vol.135, and p.2917-2929 (1989) etc. — there are some which are indicated. [for example,] Therefore, if it is this contractor, the variant used by this invention based on these well-known approaches can be obtained. Moreover, the variant used by this invention can be obtained by other variation approaches, for example, radiation irradiation etc. independent [in sucrose, a glucose, fructose, a mannitol, a sorbitol, a galactose, a maltose, Elislit, a glycerol, ethylene glycol, ethanol, etc.] as the inside of the constituent of the culture medium used for the manufacture approach of this invention, and a carbon source — or it can be used together and used. Furthermore, it can also be used, being able to add fruit juice including the starch hydrolyzate containing these things, SHITORASU molasses, beat molasses, beat juice, sugarcane juice, and citruses etc. to sucrose. Moreover, as a nitrogen source, organic or inorganic nitrogen sources, such as ammonium salt, such as an ammonium sulfate, an ammonium chloride, and ammonium phosphate, a nitrate, and a urea, can be used, or nitrogen-containing natural nutrients, such as Bact-Peptone, Bact-Soytone, Yeast-Extract, and ****, may be used. Amino acid, a vitamin, a fatty acid, a nucleic acid, 2 and 7, the 9-TORIKARUBOKISHI-1H pyrrolo [2, 3, 5]-quinoline -4, 5-dione, a sulfite waste liquor, ligninsulfonic acid, etc. may be added as organic micronutrient.

[0010] To use the auxotrophic mutant which requires amino acid etc. for growth, it is required to add the nutrient demanded supplementally. As mineral, phosphate, magnesium salt, a calcium salt, iron salt, manganese salt, cobalt salt, molybdate, red prussiate of potash, and chelate metals are used. Furthermore, the above-mentioned cellulose generation promoter can also be suitably added in a culture medium. For example, in using acetic bacteria as a production bacillus, it controls [3 thru/or 7] pH of culture to the five neighborhoods preferably. 10-40 degrees C of culture temperature are preferably performed in 25-35 degrees C. The oxygen density supplied to a culture apparatus should just be 21 - 80% desirably 1 to 100%. According to the culture approach, this contractor can choose suitably inoculation of the fungus body to the presentation rate and culture medium of each component in these culture media etc.

[0011] BC manufactured by the approach of this invention may collect fungus bodies as it is, and can perform processing which removes impurities other than the cellulose nature matter containing the fungus body further contained in this matter. independent [in heating washing of the range of 200 degrees C etc.] from processing by surfactants, such as processing by

fungus body dissolution enzymes, such as processing by bleaching agents, such as rinsing, pressurization dehydration, dilute-acid washing, alkali cleaning, sodium hypochlorite, and a hydrogen peroxide, and a lysozyme, lauryl sodium sulfate, and deoxycholic acid, and ordinary temperature, in order to remove an impurity — and it can carry out by the ability using together and an impurity can be removed from the cellulose nature matter nearly completely. thus, the thing which contains the heteropolysaccharide which used the cellulose and the cellulose as the principal chain with the cellulose nature matter as used in the field of obtained this invention and beta- the glucan of 1, 3, beta-1, and 2 grades is included. Constituents other than the cellulose in the case of heteropolysaccharide are hexose, such as a mannose, fructose, a galactose, a xylose, arabinose, rhamnose, and glucuronic acid, pentose, an organic acid, etc. In addition, polysaccharides, such as this, may be single matter and two or more sorts of polysaccharides may be intermingled by hydrogen bond etc.

[0012]

[Embodiment of the Invention] The following examples explain this invention to a detail further.

[0013]

[Example]

The value of kL a to change of a stirring rotational frequency was measured in the condition of having invested in simulation liquid [as / whose plastic viscosity is 15-20poise] to 60% of the culture apparatus which is the glass jar fermenter of whole-quantity 3L, including bacterial cellulose of 12 % of the weight of examples. Aeration of the air of 20 - 21% of oxygen tension was carried out to the simulation liquid which made dissolved oxygen concentration the saturation state about 0% by carrying out aeration of the nitrogen while rotating the gate type impeller of the various configurations shown in Table 1 next, and the dissolved oxygen concentration which goes up by this was measured using the dissolved oxygen electrode. The obtained result is shown in drawing 2 .

[0014]

[Table 1]

門型羽根	d／D	d (P) ／D
標準 (Std.)	0. 5	0. 5
WGSP	0. 65	0. 5
WGWP	0. 65	0. 65
$WG_{ex}WP$	0. 65	0. 8

d/D=(wing diameter in the grid section)/(tub bore) d (P) /D(wing diameter in the bottom section)/(tub bore)

[0015] Although kL a is calculated from the aforementioned (several 1) formula, simple, dissolved oxygen concentration is measured every 5 - 30 seconds, and kL a is calculated by the following formulas from the dissolved oxygen concentration DO 1 in time amount t1, and the dissolved oxygen concentration DO 2 in time amount t2.

((DO2-DO1)/(t2-t1))/(C* -(DO1+DO2)/2)

Unit (/hr) (however, Cin formula * dissolved oxygen concentration [**** / oxygen tension / of air bubbles])

[0016] The manufacture approach of this invention was enforced on two or less-example conditions. The BPR3001 A share (finishing [the deposition on June 12, Heisei 7] trust number FERM P-14982) which is a variant obtained from 2001 shares of BPR, and is a high-polymer cellulose production bacillus was cultivated on condition that the following.

Culture condition: The culture medium sterilized and used the CSL-Fru culture medium (refer to [Table 2, Table 3, and] Table 4) for the culture apparatus equipped with various kinds of gate type wings shown in Table 1 within the jar fermenter using 50L ** jar fermenter. Watch volume is 30L and quantity of airflow is a part for 15L/. Inoculation of the fungus liquid cultivated using the roux flask or the conical flask was carried out, and it cultivated for about 35 hours, keeping it warm at 30 degrees C. The oxygen density under aeration and exhaust air was measured using the online oxygen analyzer. The obtained result is shown in drawing 3 .

[0017]

[Table 2]

培地組成

CSL−Fru

フルクトース	7．0	(%)
KH_2PO_4	0．1	
$MgSO_4 \cdot 7H_2O$	0．25	
$(NH_4)_2SO_4$	3．3	
ビタミン混合液	1．0	
塩類混合液	1．0	
CSL（コーンステープリカー）	4．0	
pH	5．0	

[0018]
[Table 3]
Vitamin mixture Compound mg/L An inositol 200 Niacin 40 Pyridoxine HCl 40 Thiamine HCl 40 Calcium pantothenate 20 Riboflavin 20 P-aminobenzoic acid 20 Leaf Acid 0.2 biotins 0.2 [0019]
[Table 4]
Salts mixed liquor ferric ammonium citrate 1.5 g/L calcium chloride 1.5 g/L ammonium molybdate 0.1 g/L zinc-sulfate 7 monohydrate 0.2 g/L manganese-sulfate 4 monohydrate 0.1 g/L copper-sulfate 5 monohydrate 2 mg/L [0020] In addition, among drawing 3 , after BC accumulated dose (g/L) accumulated and rinsed the solid after culture termination and in culture medium and removed the culture-medium component, in 1NNaOH water solution, it was processed for 20 minutes and removed 80 degrees C of fungus bodies. Furthermore, after rinsing a generation cellulose until the penetrant remover became near neutrality, it asked by carrying out a vacuum drying at 80 degrees C for 12 hours, and measuring dry weight.

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TECHNICAL FIELD

[Field of the Invention] This invention cultivates the fungus body belonging to the microorganism (henceforth a "cellulose production bacillus") which has the capacity to produce the cellulose nature matter using the stirring equipment equipped with the gate type wing which is so-called "gate type wing", and has the description in the configuration which has opening which forms a grid in a monotonous aerofoil, and this equipment, and relates to the approach of manufacturing the cellulose nature matter (henceforth "bacterial cellulose", or "BC").

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PRIOR ART

[Description of the Prior Art] since BC (bacterial cellulose) is edible, and is used in the food field and also it is excellent in drainage system dispersibility — maintenance of the viscosity of food, cosmetics, or a coating, and a food raw material — there is industry top utility value as strengthening of the ground, maintenance of moisture, the improvement in food stability, a low-calorie-content additive, or an emulsification stabilization assistant. BC is characterized by the fragment width of face of fibril being small about 2 figures compared with the cellulose manufactured from wood pulp etc. Therefore, the disaggregation object of BC has various kinds of industrial applications as a reinforcing agent for a macromolecule, especially drainage system macromolecules based on the structural physical description which microfibril requires. Since a high modulus of elasticity in tension is shown, the outstanding mechanical characteristic based on the structural description of microfibril is expected, and the matter which solidified such a cellulose nature disaggregation object the shape of paper and in the shape of solid has the application as various industrial materials.
[0003] About the manufacture approach of BC, JP,62-265990,A, JP,63-202394,A, JP,6-43443,B, etc. have the publication about the manufacture approach of BC. It consists of a carbon source, a peptone, a yeast extract, sodium phosphate, and a citric acid as a nutrition culture medium made suitable in case a cellulose production bacillus is cultivated. Schramm/Hestrin The culture medium (Schramm et al., J.General Biology, II, pp.123-129, and 1954) is known. Moreover, it is a cellulose generation promoter according to the specific nutrient in a culture medium to such a nutrition culture medium. Add an inositol, phytic acid, a pyrrolo quinoline quinone (PQQ) (JP,5-1718,B; Mitsuo Takai, Japan Technical Association of the Pulp and Paper Industry, the 42nd volume, No. 3, the 237-244th page), etc., or Furthermore, it is found out by adding a carboxylic acid or its salt (Japanese Patent Application No. No. 191467 [five to]), an invertase (Japanese Patent Application No. No. 331491 [five to]), and a methionine (Japanese Patent Application No. No. 335764 [five to]) that the productivity of the cellulose nature matter improves. Moreover, the method of cultivating a cellulose production bacillus under the conditions of the oxygen-transfer coefficient (kL a) of the specific range is also proposed (Japanese Patent Application No. No. 31787 [seven to]). Furthermore, the method of cultivating a cellulose production bacillus is also proposed, maintaining the internal pressure of a fermenter more than fixed (Japanese Patent Application No. No. 276408 [seven to]). Moreover, as a culture format of cultivating a microorganism, standing, shaking, or aeration spinner culture has been used conventionally. Moreover, as culture operation information, the so-called batch fermentation method, the fed-batch-fermentation method, the repetitive batch fermentation method, the continuous fermentation method, etc. have been used. In addition, as a stirring means, pump drive circulation of an impeller (impeller), an air lift fermenter, and fermentation broth, the combination of these means, etc. are used, for example. As a class of impeller, the gate type wing, the turbine blade, the helical ribbon wing, the screw wing, etc. are known.
[0004] The oxygen demand of culture is made to satisfy by aeration and stirring in a industrial general fermentation process generally. However, according to many fermentation processes, it is thought important to examine the factor which rate-limiting [of the productivity] is carried out by the oxygen supply ability of a fermenter, therefore affects oxygen supply on the occasion of culture of a microorganism. It faces that the oxygen in air moves to a fungus body by the culture system, and the oxygen transfer from air bubbles to the liquid phase is represented by the degree type.
[Equation 1] $dCL/dt = kL\,a(C^*-CL) = HkL\,a(PG-PL)$ dCL / dt : Oxygen transfer rate (mmol/L-hr)
kL a: Oxygen-transfer coefficient (hr-1)
CL : Dissolved oxygen concentration in culture medium (mmol/L)
C* : Dissolved oxygen concentration [**** / oxygen tension / of air bubbles] (mmol/L)
H : Henry constant PG : Oxygen tension in a gaseous phase (it will rise, if it pressurizes)
PL : Oxygen tension in the liquid phase

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] Now, the equipment equipped with the gate type wing which unified the bottom paddle section and the grid section as a stirred tank excellent in various stirring properties from the former is the brand name "the Max blend" (Sumitomo Heavy Industries, Ltd.), and it is **********. However, there is no example which the property which was excellent in this stirred tank was evaluated using the low simulation liquid of non-Newton nature like a carboxymethyl cellulose (CMC), and was actually evaluated with the high liquid of non-Newton nature like BC. When the non-Newton nature of a solution is approximated with the exponential-function model (Power Law model) shown below It is expressed with Power Law Index (n), and change of the apparent viscosity to an average shear rate can say greatly that the non-Newton nature is high, so that this value is small.
[Equation 2]

$$\eta_{ap} = K \mid \dot{\gamma} \mid^{(n-1)}$$

etaap is an apparent viscosity and K. consistency index, [External Character 1]

$\dot{\gamma}$

a ****** shear rate and n — Power law index it is . n determines that the variation in K in each shear conditions becomes min. Incidentally, to 0.8, xanthan gum of BC is [this (n) value / CMC] very as small as 0.1 to 0.3, and the suspension or the culture medium of BC is understood that the non-Newton nature is high.
[0006] Although are desirable, therefore it is generally considered that the large-sized wing is suitable in mixing by the high fluid of the non-Newton nature that the distance of a fluid and a wing is small since change of the apparent viscosity to a shear is large, a large-sized wing has the weak shearing force over consumption power, and to be unsuitable is considered by the shear of air bubbles required for oxygen transfer. Moreover, depending on the discharge flow which the shear of the air bubbles in the bottom paddle section near a sparger or the bottom turbine section is important, and is too strong although it is also expectable that the whole fluidity improves by the discharge flow of this part, the lump of air arises near the wing, and we are anxious also about possibility of reducing a fluidity conversely. In order to raise oxygen transfer in the high fluid of the non-Newton nature until now, there is no example which examined the wing configuration near the sparger. When stirring equipment equipped with the gate type wing which carried out the specific configuration was used on the occasion of aeration stirring to for example, BC suspension or BC culture medium based on the above-mentioned recognition as a result of research of the oxygen transfer in the high fluid of the non-Newton nature, and production by fermentation, this invention person etc. found out that a high oxygen capacity coefficient (kL a) was obtained, found out that high productivity was acquired also in culture, and completed this invention.

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MEANS

[Means for Solving the Problem] That is, this invention relates to stirring equipment equipped with the gate type wing by which the ratio (d/D) to the tub bore of the wing diameter in the grid section is characterized by being 0.65 or more preferably 0.6 or more. The side face of the grid section may incline and it considers as the ratio of the wing diameter of the grid section in the minimum width of face in that case. In the gate type wing of this invention, it is [direction] desirable and the bottom paddle section or the bottom turbine section has a more desirable thing with those wing diameters (d (P)) smaller than a wing diameter [in / it is uniting with the grid section and / the grid section] (d). The stirring equipment of this invention can be especially used advantageously, in case aeration spinner culture of the cellulose production bacillus is carried out when stirring the high fluid of the non-Newton nature for example. In addition, this contractor can choose suitably the rate that the configuration and the number of the point of others about the structure and the configuration of the gate type wing of this invention, for example, the configuration and number of a grid, the bottom paddle section, or the bottom turbine sections, and a paddle aspect product occupy, the thickness of an aerofoil, etc., according to the purpose etc. Moreover, it faces enforcing this invention approach and, in addition to above-mentioned culture format and culture operation information, "said approach characterized by to be the manufacture approach of the cellulose nature matter of circulating the culture medium which contains a fungus body among decollators, such as a culture apparatus, a floatation unit, and a wedge filter, and to separate the cellulose nature matter which is a product from a fungus body and culture medium in this decollator" indicated by Japanese Patent Application No. No. 192287 [six to] can also be taken.
[0008] The cellulose production bacillus used in this invention For example, Acetobacter xylinum subsp. sucrofermentans represented by 2001 shares of BPR (Acetobacter xylinum subsp.sucrofermentans), Acetobacter xylinum () [Acetobacter] xylinum ATCC23768, Acetobacter xylinum ATCC23769, Acetobacter pasteurianus (A.pasteurianus) ATCC10245, Acetobacter xylinum ATCC14851, Acetobacter xylinum ATCC11142 To and the acetic bacteria of Acetobacter xylinum ATCC10821 grade and others Agrobacterium, Rhizobium, the Sarcina, Pseudomonas, They are the various variants invented by Achromobacter, Alcaligenes, an Aerobacter group, an azotobacter group, and the ZUGUREA group list by carrying out variation processing of them by the well-known method of using NTG (nitrosoguanidine) etc. In addition, 2001 shares of BPR is deposited with the Ministry of International Trade and Industry National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology patent microorganism deposition pin center,large on February 24, Heisei 5 (trust number FERM P-13466), and the management of it is transferred to the deposition (trust number FERM BP-4545) based on Budapest Treaty about international acknowledgement of the deposition on a patent procedure on February 7, 1994 after that.
[0009] the chemical variation art using variation agents, such as NTG, — Bio Factors, Vol.1, and p.297-302 — and (1988) — J.Gen.Microbiol, Vol.135, and p.2917-2929 (1989) etc. — there are some which are indicated. [for example,] Therefore, if it is this contractor, the variant used by this invention based on these well-known approaches can be obtained. Moreover, the variant used by this invention can be obtained by other variation approaches, for example, radiation irradiation etc. independent [in sucrose, a glucose, fructose, a mannitol, a sorbitol, a galactose, a maltose, Elislit, a glycerol, ethylene glycol, ethanol, etc.] as the inside of the constituent of the culture medium used for the manufacture approach of this invention, and a carbon source — or it can be used together and used. Furthermore, it can also be used, being able to add fruit juice including the starch hydrolyzate containing these things, SHITORASU molasses, beat molasses, beat juice, sugarcane juice, and citruses etc. to sucrose. Moreover, as a nitrogen source, organic or inorganic nitrogen sources, such as ammonium salt, such as an ammonium sulfate, an ammonium chloride, and ammonium phosphate, a nitrate, and a urea, can be used, or nitrogen-containing natural nutrients, such as Bact-Peptone, Bact-Soytone, Yeast-Extract, and ****, may be used. Amino acid, a vitamin, a fatty acid, a nucleic acid, 2 and 7, the 9-TORIKARUBOKISHI-1H pyrrolo [2, 3, 5]-quinoline -4, 5-dione, a sulfite waste liquor, ligninsulfonic acid, etc. may be added as organic micronutrient.
[0010] To use the auxotrophic mutant which requires amino acid etc. for growth, it is required to add the nutrient demanded supplementally. As mineral, phosphate, magnesium salt, a calcium salt, iron salt, manganese salt, cobalt salt, molybdate, red prussiate of potash, and chelate metals are used. Furthermore, the above-mentioned cellulose generation promoter can also be suitably added in a culture medium. For example, in using acetic bacteria as a production bacillus, it controls [3 thru/or 7] pH of culture to the five neighborhoods preferably. 10-40 degrees C of culture temperature are preferably performed in 25-35 degrees C. The oxygen density supplied to a culture apparatus should just be 21 - 80% desirably 1 to 100%. According to the culture approach, this contractor can choose suitably inoculation of the fungus body to the presentation rate and culture medium of each component in these culture media etc.
[0011] BC manufactured by the approach of this invention may collect fungus bodies as it is, and can perform processing which removes impurities other than the cellulose nature matter containing the fungus body further contained in this matter. independent [in heating washing of the range of 200 degrees C etc.] from processing by surfactants, such as processing by fungus body dissolution enzymes, such as processing by bleaching agents, such as rinsing, pressurization dehydration, dilute-acid washing, alkali cleaning, sodium hypochlorite, and a hydrogen peroxide, and a lysozyme, lauryl sodium sulfate, and deoxycholic acid, and ordinary temperature, in order to remove an impurity — and it can carry out by the ability using together and an impurity can be removed from the cellulose nature matter nearly completely. thus, the thing which contains the heteropolysaccharide which used the cellulose and the cellulose as the principal chain with the cellulose nature matter as used in the field of obtained this invention and beta- the glucan of 1, 3, beta-1, and 2 grades is included. Constituents other than the cellulose in the case of heteropolysaccharide are hexose, such as a mannose, fructose, a galactose, a xylose, arabinose, rhamnose, and glucuronic acid, pentose, an organic acid, etc. In addition, polysaccharides, such as this, may be single matter and

two or more sorts of polysaccharides may be intermingled by hydrogen bond etc.
[0012]
[Embodiment of the Invention] The following examples explain this invention to a detail further.

[Translation done.]

EXAMPLE

[Example]
The value of kL a to change of a stirring rotational frequency was measured in the condition of having invested in simulation liquid [as / whose plastic viscosity is 15-20poise] to 60% of the culture apparatus which is the glass jar fermenter of whole-quantity 3L, including bacterial cellulose of 12 % of the weight of examples. Aeration of the air of 20 - 21% of oxygen tension was carried out to the simulation liquid which made dissolved oxygen concentration the saturation state about 0% by carrying out aeration of the nitrogen while rotating the gate type impeller of the various configurations shown in Table 1 next, and the dissolved oxygen concentration which goes up by this was measured using the dissolved oxygen electrode. The obtained result is shown in drawing 2 .
[0014]
[Table 1]

門型羽根	d／D	d (P) ／D
標準 (Std.)	0．5	0．5
WGSP	0．65	0．5
WGWP	0．65	0．65
$WG_{ex}WP$	0．65	0．8

d/D=(wing diameter in the grid section)/(tub bore) d (P) /D(wing diameter in the bottom section)/(tub bore)
[0015] Although kL a is calculated from the aforementioned (several 1) formula, simple, dissolved oxygen concentration is measured every 5 - 30 seconds, and kL a is calculated by the following formulas from the dissolved oxygen concentration DO 1 in time amount t1, and the dissolved oxygen concentration DO 2 in time amount t2.
((DO2-DO1)/(t2-t1))/(C* -(DO1+DO2)/2)
Unit (/hr) (however, Cin formula * dissolved oxygen concentration [**** / oxygen tension / of air bubbles])
[0016] The manufacture approach of this invention was enforced on two or less-example conditions. The BPR3001 A share (finishing [the deposition on June 12, Heisei 7] trust number FERM P-14982) which is a variant obtained from 2001 shares of BPR, and is a high-polymer cellulose production bacillus was cultivated on condition that the following.
Culture condition: The culture medium sterilized and used the CSL-Fru culture medium (refer to [Table 2, Table 3, and] Table 4) for the culture apparatus equipped with various kinds of gate type wings shown in Table 1 within the jar fermenter using 50L ** jar fermenter. Watch volume is 30L and quantity of airflow is a part for 15L/. Inoculation of the fungus liquid cultivated using the roux flask or the conical flask was carried out, and it cultivated for about 35 hours, keeping it warm at 30 degrees C. The oxygen density under aeration and exhaust air was measured using the online oxygen analyzer. The obtained result is shown in drawing 3 .
[0017]
[Table 2]

培地組成

ＣＳＬ－Ｆｒｕ

フルクトース	7．0	(%)
KH_2PO_4	0．1	
$MgSO_4 \cdot 7H_2O$	0．25	
$(NH_4)_2SO_4$	3．3	
ビタミン混合液	1．0	
塩類混合液	1．0	
ＣＳＬ（コーンステープリカー）	4．0	
ｐＨ	5．0	

[0018]
[Table 3]
Vitamin mixture Compound mg/L An inositol 200 Niacin 40 Pyridoxine HCl 40 Thiamine HCl 40 Calcium pantothenate 20 Riboflavin 20 P-aminobenzoic acid 20 Leaf Acid 0.2 biotins 0.2 [0019]
[Table 4]
Salts mixed liquor ferric ammonium citrate 1.5 g/L calcium chloride 1.5 g/L ammonium molybdate 0.1 g/L zinc-sulfate 7 monohydrate 0.2 g/L manganese-sulfate 4 monohydrate 0.1 g/L copper-sulfate 5 monohydrate 2 mg/L [0020] In addition, among drawing 3 , after BC accumulated dose (g/L) accumulated and rinsed the solid after culture termination and in culture medium and removed the culture-medium component, in 1NNaOH water solution, it was processed for 20 minutes and removed 80 degrees C of fungus bodies. Furthermore, after rinsing a generation cellulose until the penetrant remover became near neutrality, it asked by carrying out a vacuum drying at 80 degrees C for 12 hours, and measuring dry weight.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

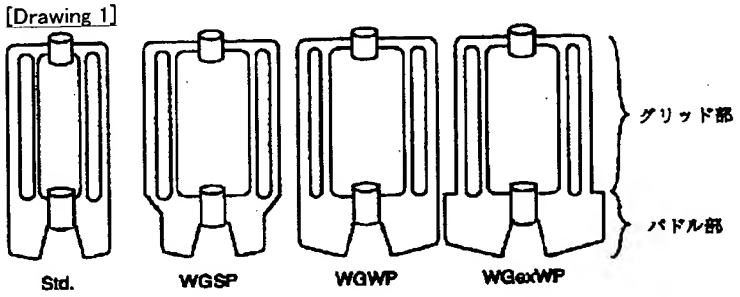
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The actual configuration of the various gate type wings shown in Table 1 is shown.
[Drawing 2] The relation between a stirring rotational frequency and kL a is shown.
[Drawing 3] Aging of BC accumulated dose at the time of using each culture apparatus is shown.

[Translation done.]

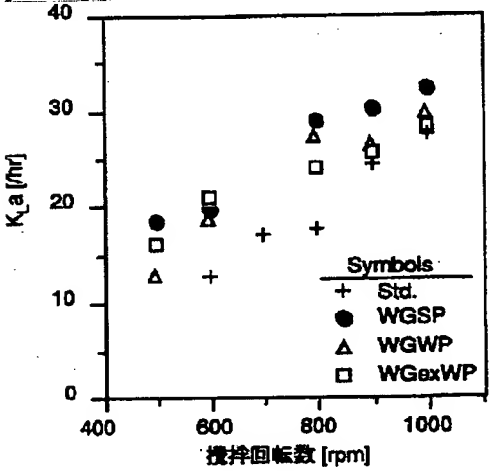
DRAWINGS

[Drawing 1]



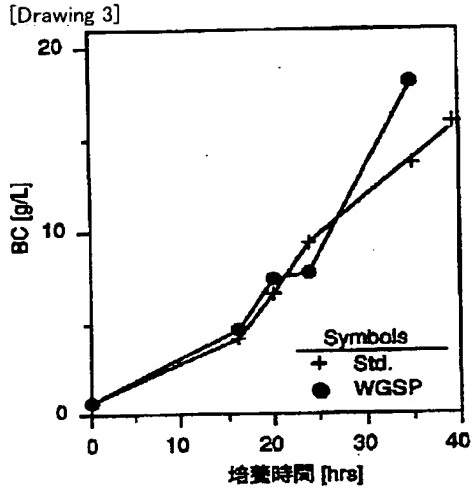
門型撹拌羽根の各種形状

[Drawing 2]



撹拌回転数とK_Laの関係

[Drawing 3]



各種培養装置を用いた場合のBC蓄積量の経時変化

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号
特開平9－220457
(43)公開日　平成９年(1997)８月26日

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ		技術表示箇所
Ｂ０１Ｆ　7/16			Ｂ０１Ｆ　7/16	Ｆ	
Ｃ０８Ｂ　15/00			Ｃ０８Ｂ　15/00		
Ｃ１２Ｍ　1/02			Ｃ１２Ｍ　1/02	Ａ	
Ｃ１２Ｐ　19/04			Ｃ１２Ｐ　19/04	Ｃ	

審査請求　未請求　請求項の数６　ＦＤ　（全６頁）

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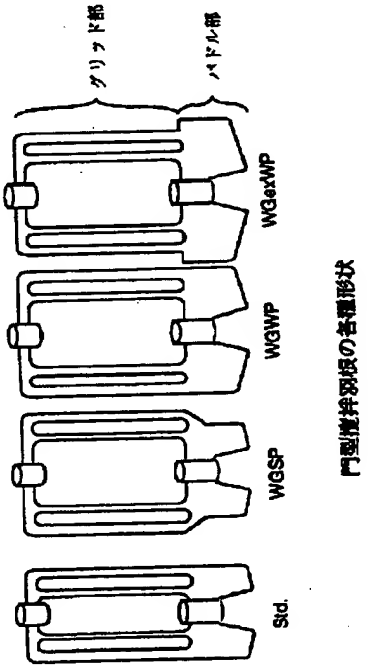
(54)【発明の名称】　高非ニュートン流体撹拌用門型羽根

(57)【要約】
【課題】　高いニュートン性を有する流体の撹拌に使用した際に、高い酸素容量係数（ｋＬａ）が得られる撹拌装置を提供すること。
【解決手段】　グリッド部に於ける翼径の槽内径に対する比が０．６以上であることを特徴とする門型羽根を備えた撹拌装置及び該装置の非ニュートン性の高い流体の撹拌への使用。



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【特許請求の範囲】
【請求項１】　グリッド部に於ける翼径の槽内径に対する比が０．６以上であることを特徴とする門型羽根を備えた撹拌装置。
【請求項２】　ボトムパドル部又はボトムタービン部に於ける翼径がグリッド部に於ける翼径よりも小さいことを特徴とする請求項１記載の門型羽根を備えた撹拌装置。
【請求項３】　非ニュートン性の高い流体の撹拌に使用する為の請求項１又は２記載の撹拌装置。
【請求項４】　セルロース生産菌の培養に使用する為の請求項１又は２記載の撹拌装置。
【請求項５】　請求項１又は２に記載の撹拌装置を発酵槽として用いてセルロース生産菌を通気撹拌培養し、セルロース性物質を製造する方法。
【請求項６】　請求項１又は２記載の撹拌装置のセルロース生産菌の培養への使用。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は、平板翼にグリッドを形成するような開口部を有する、いわゆる「門型羽根」であって、その形状に特徴を有する門型羽根を備えた撹拌装置及び該装置を用いた、セルロース性物質を生産する能力を有する微生物（以下、「セルロース生産菌」という。）に属する菌体を培養し、セルロース性物質（以下、「バクテリアセルロース」又は「ＢＣ」という。）を製造する方法に関する。
【０００２】
【従来の技術】ＢＣ（バクテリアセルロース）は可食性であり食品分野で利用されるほか水系分散性に優れているので食品、化粧品又は塗料等の粘度の保持、食品原料生地の強化、水分の保持、食品安定性向上、低カロリー添加物又は乳化安定化助剤としての産業上利用価値がある。ＢＣは木材パルプ等から製造されるセルロースに較べ、フィブリルの断片幅が２ケタ程度も小さいことを特徴とする。従って、ＢＣの離解物はミクロフィブリルのかかる構造的物理的特徴に基づき高分子、特に水系高分子用補強剤として各種の産業用用途がある。このようなセルロース性離解物を紙状または固型状に固化した物質は高い引張弾性率を示すのでミクロフィブリルの構造的特徴に基づくすぐれた機械特性が期待され、各種産業用素材としての応用がある。
【０００３】ＢＣの製造方法に関しては、特開昭６２－２６５９９０号、特開昭６３－２０２３９４号及び特公平６－４３４４３号等にＢＣの製造方法に関する記載がある。セルロース生産菌の培養を行なう際に適当とされている栄養培地としては、炭素源、ペプトン、酵母エキス、燐酸ナトリウム及びクエン酸からなる Schramm／Hestrin 培地（Schramm ら、J. General Biology, 11, pp.123～129, 1954 ）が知られている。また、このような栄養培地に、培地中の特定栄養素によるセルロース生成促進因子である、イノシトール、フィチン酸及びピロロキノリンキノン（ＰＱＱ）（特公平５－１７１８号公報；高井光男，紙パ技協誌，第４２巻，第３号，第２３７～２４４頁）等を添加したり、更には、カルボン酸又はその塩（特願平５－１９１４６７号）、インベルターゼ（特願平５－３３１４９１号）及びメチオニン（特願平５－３３５７６４号）を添加することによって、セルロース性物質の生産性が向上することが見い出されている。又、特定の範囲の酸素移動容量係数（$k_L a$）の条件下でセルロース生産菌を培養する方法も提案されている（特願平７－３１７８７号）。更に、発酵槽の内圧を一定以上に保ちながらセルロース生産菌を培養する方法も提案されている（特願平７－２７６４０８号）。また、従来より、微生物を培養する培養形式としては、静置、振盪もしくは通気撹拌培養等が用いられてきた。また、培養操作法としては、いわゆる回分発酵法、流加回分発酵法、反復回分発酵法及び連続発酵法等が使用されてきた。尚、撹拌手段としては、例えばインペラー（撹拌羽根）、エアーリフト発酵槽、発酵ブロスのポンプ駆動循環、及びこれら手段の組合せ等が使用されている。インペラーの種類としては、門型羽根、タービン羽根、ヘリカルリボン羽根及びスクリュー羽根等が知られている。

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【０００４】一般に、工業的な発酵プロセス一般に於いては、培養の酸素要求量を通気と撹拌で充足させている。しかし、多くの発酵プロセスでは発酵槽の酸素供給能で生産性が律速されており、従って、微生物の培養に際して酸素供給に影響を与える要因を検討することは重要であると考えられる。培養系で空気中の酸素が菌体に移動するに際して、気泡から液相への酸素移動は次式によって代表される。
【数１】$dC_L/dt = k_L a (C^* - C_L) = Hk_L a (P_g - P_L)$
dC_L/dt：　酸素移動速度（mmol／Ｌ・hr）
$k_L a$：　酸素移動容量係数（hr^{-1}）
C_L　：　培養液中の溶存酸素濃度（mmol／Ｌ）
C^*　：　気泡の酸素分圧と平衡な溶存酸素濃度（mmol／Ｌ）
Ｈ　：　ヘンリー定数
P_g　：　気相中の酸素分圧（加圧すると高まる）
P_L　：　液相中の酸素分圧
【０００５】
【発明が解決しようとする課題】さて、従来から、種々の撹拌特性に優れた撹拌槽として、ボトムパドル部とグリッド部を一体化した門型羽根を備えた装置が「マックスブレンド」（住友重機械工業株式会社）という商標名で各種知られている。しかしながら、この撹拌槽の優れた特性は、カルボキシメチルセルロース（ＣＭＣ）のような非ニュートン性の低い模擬液を用いて評価されたも

のであり、ＢＣのような非ニュートン性の高い液体で実際に評価された例はない。溶液の非ニュートン性は、以下に示す指数関数モデル（Power Law モデル）で近似したときの Power Law Index（ｎ）で表され、この値が小さいほど、平均剪断速度に対する見かけ粘度の変化が大きい、即ち、非ニュートン性が高いといえる。

【数２】

$$\eta_{ap} = K \mid \dot{\gamma} \mid^{(n-1)}$$

η_{ap}は見かけ粘度、Kは consistency index、

【外１】

$\dot{\gamma}$

は平均剪断速度、ｎはPower law index である。ｎは各剪断条件におけるＫのバラツキが最小になるように定める。因みに、この（ｎ）値は、ＣＭＣが０．８、キサンタンガムが０．３に対して、ＢＣは０．１と非常に小さく、ＢＣの懸濁液又は培養液は非ニュートン性が高いことが判る。

【０００６】一般に、非ニュートン性の高い流体では剪断に対する見かけ粘度の変化が大きいため、混合においては流体と羽根との距離が小さいことが望ましく、従って大型羽根が適していると考えられるが、大型羽根は消費動力に対する剪断力が弱く、酸素移動に必要な気泡の剪断には不適当であると考えられる。また、スパージャーに近いボトムパドル部またはボトムタービン部における気泡の剪断は重要であり、この部分の吐出流によって全体の流動性が向上することも期待できるが、強すぎる吐出流によっては羽根近傍に空気のかたまりが生じ、逆に流動性を低下させる可能性も懸念される。これまでに、非ニュートン性の高い流体において酸素移動を高めるためにスパージャー近傍の羽根形状を検討した例はない。本発明者等は、上記認識にもとづき、非ニュートン性の高い流体における酸素移動と発酵生産の研究の結果、特定の形状をした門型羽根を備えた撹拌装置を、例えばＢＣ懸濁液やＢＣ培養液への通気撹拌に際して使用すると、高い酸素容量係数（ｋ，ａ）が得られることを見いだし、培養においても高い生産性が得られることを見いだし、本発明を完成させた。

【０００７】

【課題を解決するための手段】即ち、本発明は、グリッド部に於ける翼径の槽内径に対する比（ｄ／Ｄ）が０．６以上、好ましくは０．６５以上であることを特徴とする門型羽根を備えた撹拌装置に係わる。グリッド部の側面が傾斜しても良く、その場合は、最小幅に於けるグリッド部の翼径の比とする。本発明の門型羽根に於いては、ボトムパドル部又はボトムタービン部はグリッド部と一体化している方が好ましく、それらの翼径（ｄ（Ｐ））がグリッド部に於ける翼径（ｄ）よりも小さいものがより好ましい。本発明の撹拌装置は、非ニュートン性の高い流体を撹拌するような場合、例えば、セルロース生産菌を通気撹拌培養する際に、特に有利に使用することができる。尚、本発明の門型羽根の構造・形状に関するその他の点、例えば、グリッドの形状・数、ボトムパドル部ないしボトムタービン部の形状・数、パドル部面積の占める割合及び翼の厚さ等は、当業者が目的等に応じて適宜選択し得る。又、本発明方法を実施するに際しては、前述の培養形式・培養操作法に加えて、特願平６－１９２２８７号に記載されている「培養装置と浮上分離装置及びウェッジフィルター等の分離装置の間で菌体を含む培養液を循環させるセルロース性物質の製造方法であって、該分離装置に於いて、生産物であるセルロース性物質を菌体及び培養液から分離することを特徴とする、前記方法」を採ることもできる。

【０００８】本発明において使用されるセルロース生産菌は、例えば、ＢＰＲ２００１株に代表されるアセトバクター・キシリナム・サブスピーシーズ・シュクロファーメンタンス（Acetobacter xylinum subsp. sucrofermentans）、アセトバクター・キシリナム（Acetobacter xylinum）ＡＴＣＣ２３７６８、アセトバクター・キシリナムＡＴＣＣ２３７６９、アセトバクター・パスツリアヌス（A. pasteurianus）ＡＴＣＣ１０２４５、アセトバクター・キシリナムＡＴＣＣ１４８５１、アセトバクター・キシリナムＡＴＣＣ１１１４２及びアセトバクター・キシリナムＡＴＣＣ１０８２１等の酢酸菌、その他に、アグロバクテリウム属、リゾビウム属、サルシナ属、シュードモナス属、アクロモバクター属、アルカリゲネス属、アエロバクター属、アゾトバクター属及びズーグレア属並びにそれらをＮＴＧ（ニトロソグアニジン）等を用いる公知の方法によって変異処理することにより創製される各種変異株である。尚、ＢＰＲ２００１株は、平成５年２月２４日に通商産業省工業技術院生命工学工業技術研究所特許微生物寄託センターに寄託され（受託番号ＦＥＲＭ　Ｐ－１３４６６）、その後１９９４年２月７日付で特許手続上の寄託の国際的承認に関するブダペスト条約に基づく寄託（受託番号ＦＥＲＭ　ＢＰ－４５４５）に移管されている。

【０００９】ＮＴＧ等の変異剤を用いての化学的変異処理方法には、例えば、Bio Factors,Vol. 1, p.297－302 (1988)及び J. Gen. Microbiol, Vol. 135, p.2917－2929(1989) 等に記載されているものがある。従って、当業者であればこれら公知の方法に基づき本発明で用いる変異株を得ることができる。また、本発明で用いる変異株は他の変異方法、例えば放射線照射等によっても得ることができる。本発明の製造方法に用いる培地の組成物中、炭素源としてはシュクロース、グルコース、フラクトース、マンニトール、ソルビトール、ガラクトース、マルトース、エリスリット、グリセリン、エチレングリコール、エタノール等を単独或いは併用して使用することができる。更にはこれらのものを含有する澱粉水解

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物、シトラスモラセス、ビートモラセス、ビート搾汁、サトウキビ搾汁、柑橘類を始めとする果汁等をシュクロースに加えて使用することもできる。　また、窒素源としては硫酸アンモニウム、塩化アンモニウム、リン酸アンモニウム等のアンモニウム塩、硝酸塩、尿素等有機或いは無機の窒素源を使用することができ、或いはＢａｃｔ－Ｐｅｐｔｏｎｅ、Ｂａｃｔ－Ｓｏｙｔｏｎｅ、Ｙｅａｓｔ－Ｅｘｔｒａｃｔ、豆濃などの含窒素天然栄養源を使用してもよい。有機微量栄養素としてアミノ酸、ビタミン、脂肪酸、核酸、２，７，９－トリカルボキシ－１Ｈピロロ〔２，３，５〕－キノリン－４，５－ジオン、亜硫酸パルプ廃液、リグニンスルホン酸等を添加してもよい。

【００１０】生育にアミノ酸等を要求する栄養要求性変異株を使用する場合には、要求される栄養素を補添することが必要である。無機塩類としてはリン酸塩、マグネシウム塩、カルシウム塩、鉄塩、マンガン塩、コバルト塩、モリブデン酸塩、赤血塩、キレート金属類等が使用される。更に、前述のセルロース生成促進因子を適宜培地中に添加することもできる。例えば、酢酸菌を生産菌として用いる場合には、培養のｐＨは３ないし７に、好ましくは５付近に制御する。培養温度は１０～４０℃、好ましくは２５～３５℃の範囲で行う。培養装置に供給する酸素濃度は１～１００%、望ましくは２１～８０%であれば良い。これら培地中の各成分の組成割合及び培地に対する菌体の接種等は培養方法に応じて当業者が適宜選択し得るものである。

【００１１】本発明の方法によって製造されるＢＣは菌体はそのまま回収してもよく、さらに本物質中に含まれる菌体を含むセルロース性物質以外の不純物を取り除く処理を施すことが出来る。不純物を取り除くためには、水洗、加圧脱水、希酸洗浄、アルカリ洗浄、次亜塩素酸ソーダ及び過酸化水素などの漂白剤による処理、リゾチ＊

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＊ームなどの菌体溶解酵素による処理、ラウリル硫酸ソーダ、デオキシコール酸などの界面活性剤による処理、常温から２００℃の範囲の加熱洗浄などを単独及び併用して行い、セルロース性物質から不純物をほぼ完全に除去することができる。このようにして得られた本発明でいうセルロース性物質とは、セルロース及び、セルロースを主鎖としたヘテロ多糖を含むもの及びβ－１，３、β－１，２等のグルカンを含むものである。ヘテロ多糖の場合のセルロース以外の構成成分はマンノース、フラクトース、ガラクトース、キシロース、アラビノース、ラムノース、グルクロン酸等の六炭糖、五炭糖及び有機酸等である。尚、これ等の多糖が単一物質である場合もあるし２種以上の多糖が水素結合等により混在してもよい。

【００１２】

【発明の実施の形態】以下の実施例により、本発明をさらに詳細に説明する。

【００１３】

【実施例】

実施例１

２重量%のバクテリアセルロースを含み、かつ塑性粘度が１５～２０ポイズであるような模擬液を全量３Ｌのガラス製ジャーファーメンターである培養装置の６０%に張り込んだ状態で撹拌回転数の変化に対するｋｌ ａの値を測定した。表１に示す各種形状の門型撹拌羽根を回転させながら窒素を通気することにより溶存酸素濃度をおよそ０%飽和状態とした模擬液に、次に酸素分圧２０～２１%の空気を通気し、これによって上昇する溶存酸素濃度を溶存酸素電極を用いて測定した。得られた結果を図２に示す。

【００１４】

【表１】

門型羽根	d／D	d（P）／D
標準（Std.）	0．5	0．5
WGSP	0．65	0．5
WGWP	0．65	0．65
$WG_{0.1}WP$	0．65	0．8

d／D＝（グリッド部に於ける翼径）／（槽内径）

d（P）／D（ボトム部に於ける翼径）／（槽内径）

【００１５】ｋｌ ａは前記（数１）式より求められるが、簡便には、５～３０秒毎に溶存酸素濃度を測定し、時間ｔ１での溶存酸素濃度ＤＯ１と時間ｔ２での溶存酸素濃度ＤＯ２から以下の式でｋｌ ａを求める。

（（DO2－DO1）／（t2－t1））／（C＊－（DO1＋DO2）／2）

、単位（／hr）　（但し、式中C＊は気泡の酸素分圧と平衡な溶存酸素濃度）

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【００１６】実施例２

以下の条件で、本発明の製造方法を実施した。ＢＰＲ２００１株から得られた変異株であって高重合度セルロース生産菌であるＢＰＲ３００１Ａ株（平成７年６月１２日付寄託済、受託番号ＦＥＲＭ　Ｐ－１４９８２）を以下の条件で培養した。

培養条件：表１に示した各種の門型羽根を備えた培養装置には５０Ｌ容ジャーファーメンターを用い、培地はＣＳＬ－Ｆｒｕ培地（表２、表３及び表４参照）をジャー＊

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＊ファーメンター内で殺菌して用いた。張り込み液量は３０Ｌ、通気量は１５Ｌ／分である。ルーフラスコやコニカル・フラスコを用いて培養した菌液を植菌し、３０℃に保温しながら約３５時間培養した。通気中及び排気中の酸素濃度はオンライン酸素濃度計を用いて測定した。得られた結果を図３に示す。

【００１７】

【表２】

培地組成

ＣＳＬ－Ｆｒｕ

フルクトース	７．０　(%)
KH_2PO_4	０．１
$MgSO_4 \cdot 7H_2O$	０．２５
$(NH_4)_2SO_4$	３．３
ビタミン混合液	１．０
塩類混合液	１．０
ＣＳＬ（コーンステープリカー）	４．０
ｐＨ	５．０

【００１８】

※　※【表３】

ビタミン混合物

化合物	ｍｇ／Ｌ
イノシトール	２００
ナイアシン	４０
ピリドキシンＨＣｌ	４０
チアミンＨＣｌ	４０
パントテン酸カルシウム	２０
リボフラビン	２０
ｐ－アミノ安息香酸	２０
葉　酸	０．２
ビオチン	０．２

【００１９】

【表４】

塩類混合液

クエン酸鉄アンモニウム	１．５ｇ／Ｌ
塩化カルシウム	１．５ｇ／Ｌ
モリブデン酸アンモニウム	０．１ｇ／Ｌ
硫酸亜鉛７水塩	０．２ｇ／Ｌ
硫酸マンガン４水塩	０．１ｇ／Ｌ
硫酸銅５水塩	２ｍｇ／Ｌ

【００２０】尚、図３中、ＢＣ蓄積量（ｇ／Ｌ）は、培養終了後、培養液中の固形物を集積し、水洗して培地成分を除去した後、１ＮＮａＯＨ水溶液中で８０℃、２０分間処理して菌体を除去した。さらに、洗浄液が中性付近になるまで生成セルロースを水洗した後、８０℃で１２時間真空乾燥して乾燥重量を測定することで求めた。

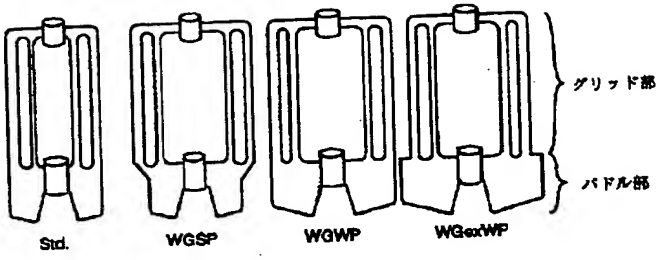
【図面の簡単な説明】

【図１】　表１に示した各種門型羽根の実際の形状を示す。

【図２】　撹拌回転数と$k_L a$との関係を示す。

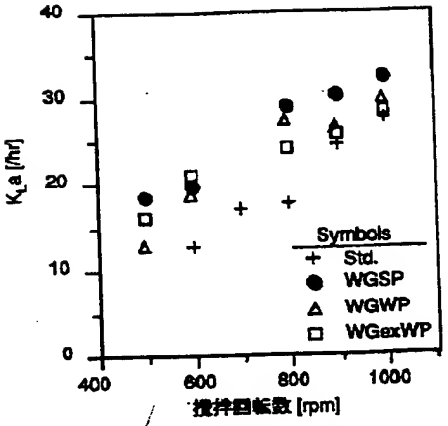
【図３】　各培養装置を用いた場合のＢＣ蓄積量の経時変化を示す。

【図１】



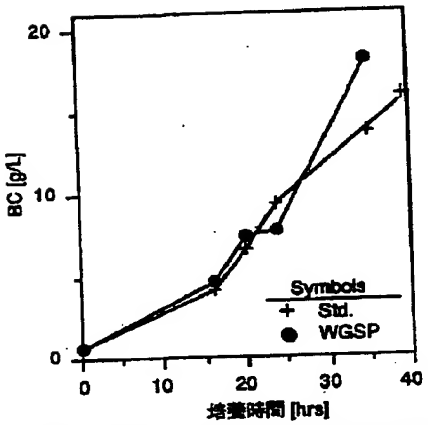
門型攪拌羽根の各種形状

【図２】



攪拌回転数とK_Laの関係

【図３】



各種培養装置を用いた場合のBC蓄積量の経時変化